

WHAT IS CLAIMED IS:

1. A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:

- (a) collecting whole blood;
- (b) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
- (c) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA;
- (d) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA; and
- (e) quantifying the specific mRNA.

2. The method of Claim 1, wherein an anticoagulant is administered to the whole blood prior to collection of leukocytes.

3. The method of Claim 1, wherein heparin is administered to the whole blood prior to collection of leukocytes.

4. The method of Claim 1, wherein the whole blood is frozen prior to filtration.

5. The method of Claim 1, wherein the filter membrane is attached to a multi-well filter plate.

6. The method of Claim 1, wherein the filter membrane is a PBT fibrous membrane.

7. The method of Claim 5, wherein the leukocytes are captured on a plurality of filter membranes layered together.

8. The method of Claim 1, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.

9. The method of Claim 8, additionally comprising drying the filter membrane.

10. The method of Claim 9, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

11. The method of Claim 1, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

12. The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

13. The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

14. The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

15. The method of Claim 1, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.

16. The method of Claim 1, wherein the mRNA quantified is β -actin mRNA.

17. The method of Claim 1, wherein the mRNA quantified is CD4 mRNA.

18. The method of Claim 1, wherein the mRNA of a translocation gene involved in leukemia is quantified.

19. The method of Claim 1, wherein the mRNA of cancer-specific genes from micrometastatic cancer is quantified.

20. The method of Claim 1, wherein virus-derived mRNA from infected white blood cells is quantified.

21. The method of Claim 20, wherein the quantified virus-derived mRNA is HIV

22. The method of Claim 21, wherein the quantification of HIV mRNA is used to diagnose HIV.

23. The method of Claim 20, wherein the quantified virus-derived mRNA is CMV.

24. The method of Claim 23, wherein the quantification of virus-derived mRNA is used to diagnose CMV.

25. The method of Claim 20, wherein the quantification of virus-derived mRNA is used to monitor blood banks for the presence of viral diseases.

26. The method of Claim 20, wherein the quantification of virus-derived mRNA is used to study anti-viral drug sensitivity.

27. The method of Claim 1, wherein the mRNA of apoptosis genes involved in leukemia is quantified.

28. The method of Claim 1, wherein the mRNA of cytokines is quantified.

29. The method of Claim 1, wherein the quantification of mRNA is used to test side effects of anti-cancer drugs on white blood cells.

30. The method of Claim 1, wherein the mRNA of DNA-repair genes is quantified.

31. The method of Claim 30, wherein the quantification of mRNA of DNA-repair genes is used to test the sensitivity of DNA-repair genes to radiation.

32. The method of Claim 1, wherein the mRNA of allergen response genes is quantified.

33. The method of Claim 32, wherein the quantification of mRNA of allergen response genes is used to test allergen stimulation.

34. The method of Claim 1, wherein the mRNA of donor cell-mediated cytokines is quantified.

35. The method of Claim 34, wherein the quantification of mRNA of donor cell-mediated cytokines is used to test transplant rejection.

36. A high throughput mRNA quantification device, comprising:

(a) a multi-well plate, said multi-well plate comprising:

i) a plurality of sample-delivery wells;

ii) a leukocyte-capturing filter underneath said wells;

iii) an mRNA capture zone underneath said filter, said mRNA capture zone having oligo(dT)-immobilized thereon; and

(b) a vacuum box adapted to receive said plate to create a seal between said plate and said box.

37. The device of Claim 36, said vacuum box being adapted to receive a source of vacuum.

38. The device of Claim 36, said vacuum box being made of plastic.

39. The device of Claim 36, wherein said seal comprises a plastic-based gasket placed below the multi-well plate.

40. The device of Claim 36, wherein a multi-well supporter is inserted between the vacuum box and the multi-well plate.

41. The device of Claim 36, wherein the leukocytes are captured on a plurality of filter membranes layered together.

42. A lysis buffer for high throughput mRNA quantification, comprising:

(a) a sufficient concentration of detergent to lyse a cytoplasmic membrane;

(b) a sufficient concentration of salt that the stringency does not exceed that of 4X SSC;

(c) a buffer to maintain pH in the range of 7.0-8.0;

(d) 1.4-1.75 M guanine thiocyanate; and

(e) 200 µg/ml -20 mg/ml proteinase K.

43. The lysis buffer of Claim 42, wherein the concentration of detergent is sufficient to lyse both cytoplasmic and nuclear membranes.

44. The lysis buffer of Claim 42, wherein the detergent comprises a plurality of detergents.

45. The lysis buffer of Claim 42, wherein the detergent comprises 0.1-2% IGEPAL CA-630.

46. The lysis buffer of Claim 42, wherein the detergent comprises 0.05-2% N-Lauroylsarcosine.

47. The lysis buffer of Claim 42, wherein the buffer is sufficient to maintain pH in the range of 7.4-8.0.

48. The lysis buffer of Claim 47, wherein the buffer comprises 1 mM-100 mM Tris HCL.

49. The lysis buffer of Claim 42, comprising about 1.6 M to about 1.7 M guanidine thiocyanate.

50. The lysis buffer of Claim 42, comprising 200 µg/ml -1.0 mg/ml proteinase K

51. The lysis buffer of Claim 42, comprising 200 µg/ml -500µg/ml proteinase K.

52. The lysis buffer of Claim 42, further comprising a chelating agent in an amount sufficient to chelate Mg^{2+} and Ca^{2+} .

53. The lysis buffer of Claim 52, wherein the chelating agent comprises 0.1-5 mM EDTA.

54. The lysis buffer of Claim 42, further comprising 0.1-10% 2-mercaptoethanol.
55. The lysis buffer of Claim 42, further comprising DNA.
56. The lysis buffer of Claim 55, wherein the DNA comprises 10 mg/ml sonicated salmon sperm DNA.
57. The lysis buffer of Claim 42, further comprising tRNA.
58. The lysis buffer of Claim 57, wherein the tRNA comprises 10 mg/ml E. Coli tRNA.
59. A high throughput mRNA quantification kit, comprising:
 - (a) the high throughput mRNA quantification device of Claim 36;
 - (b) a hypotonic buffer;
 - (c) ethanol; and
 - (d) a lysis buffer.
60. The kit of Claim 59, wherein the lysis buffer comprises 1.4-1.75 M guanine thiocyanate; and 200 µg/ml -20 mg/ml proteinase K.
61. The kit of Claim 60, wherein the lysis buffer further comprises sufficient detergent to lyse a cytoplasmic membrane; sufficient salt that the stringency does not exceed that of 4X SSC; and a buffer to maintain pH in the range of 7.0-8.0.
62. The kit of Claim 60, wherein the lysis buffer further comprises sufficient salt that the stringency does not exceed that of 4X SSC.
63. The kit of Claim 60, wherein the lysis buffer further comprises sufficient buffer to maintain pH in the range of 7.0-8.0.
64. A method of lysing cells, comprising exposing cells to the lysis buffer of Claims 42.